Studies on Entomopathogenic Fungi to Manage Pests of Litchi

Synopsis

Submitted in partial fulfillment of the requirements
for the award of Ph.D degree in Zoology,
Under the Faculty of Science,
Lalit Narayan Mithila University,
Darbhanga

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Introduction

Litchi (*Litchi chinensis* Sonn.) is one of the most important subtropical fruit trees of the family Sapindaceae. India is the second largest producer of litchi in the world after China with an area and production of 84,170 and 585,300 tones, respectively during 2013-14. It is also cultivated in Thailand, Australia, South Africa, Madagascar, and Florida in US. In India, Bihar, West Bengal, Jharkhand and Assam accounts for 64.2% of the total litchi production in the country. The other litchi producing states of India are Chhattisgarh, Uttarakhand, Punjab, Odisha, Tripura, Himachal Pradesh and union territory of Jammu & Kashmir. In Bihar, important litchi growing districts are Muzaffarpur, Vaishali, Sitamarhi, West Champaran, East Champaran, Darbhanga, and Samastipur.

Litchi cultivation faces significant challenges from insect pests. A variety of insects and mite species are important pests of litchi trees and fruits at various growth stages, resulting in significant yield losses. Currently, litchi fruit borer, shoot borer, litchi looper, stink bug and flower webbers are major pests of litchi limiting healthy and profitable litchi production (Kumar et al. 2014a; 2014b; Srivastava et al. 2019; 2020)

Currently, growers predominantly rely on chemical pesticides for managing the pests of litchi. However, the widespread use of synthetic pesticides has adverse effects in the agro-ecosystem. These include diminishing crop productivity, harming human health, and negatively impacting diverse beneficial non-target organisms. Hence, exploring alternative pest management strategies is crucial for minimizing these impacts. There is a pressing need for biopesticides, which offer eco-friendly, safe, highly efficient, and rapidly degradable characteristics. Biopesticides derived from microbiomes offer a non-toxic method for controlling insect pest populations. Biocontrol agents include wide range of organisms, such

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as viruses, bacteria, fungi, nematodes, protozoa. Among these, entomopathogenic fungi (EPF) is a potential biocontrol agent, offering an environmentally friendly alternative to chemical pesticides. They offer several advantages, including broad host range, simple production methods, and potent virulence.

'Entomopathogenic fungi' are type of fungi that can kill or seriously disable insects. These amazing fungi attach to the external body surface of insects in the form of microscopic spores, usually asexual, mitosporic spores also called 'conidia'. Under the right conditions of temperature and high humidity, these spores germinate, grow as hyphae, and colonize the insect's cuticle. They bore through the cuticle via enzymatic hydrolysis, reaching the insect's body cavity (hemocoel). Within the host body, the fungal cells proliferate, leading to the insect's demise. New spores are formed if environmental conditions are favorable. High humidity is usually required for sporulation (Gul et al. 2014).

Entomopathogenic fungi (EPF) are parasitic microorganisms with the ability to infect and kill arthropods. They penetrate through the insect's cuticle to obtain nutrients for their growth and reproduction, a process requiring both mechanical pressure and enzymatic degradation. Once inside, they degrade the host cuticle and proliferate within the insect's hemolymph as hyphal bodies, secreting toxins that induce the death of the host insects(Butt et al. 2013). During the pathogenesis process, various mycotoxins are generated, including Beauvericin (produced by *Beauveria bassiana*), Beauverolides (from *Verticillium lecanii*), Bassianolide (associated with *Paecilomyces* spp.), and Destruxins A, B, C, D, E, F (produced by *Metarhizium anisopliae*). These mycotoxins are toxic to insects, contributing to the pathogenicity of fungi (Irsad et al. 2023). Entomophthoralean group of fungi are obligate

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insect pathogens that kill their hosts through tissue colonization without the use of toxins (Pell et al. 2001).

Entomopathogenic fungi include taxa from several main fungal groups and do not form a monophyletic group. Some examples of entomopathogenic fungi that play a crucial role in managing insect pests are:

- *Beauveria bassiana*: This fungus, belonging to the order Hypocreales (Ascomycota), is widely used as a biopesticide. It infects insects by attaching to their external body surface and subsequently colonizing their cuticle. *Beauveria bassiana* has both asexual (anamorph) and sexual (teleomorph) phases (Gul et al. 2014, Sharma et al. 2021).
- Metarhizium: Another member of the Hypocreales order, *Metarhizium* species are effective entomopathogens. They can be grown on starch-rich substrates like rice or wheat grains. *Metarhizium* spp. are under scrutiny for their traits favouring use as biopesticides (Gul et al. 2014, Deka et al 2021)
- *Cordyceps*: These fungi exhibit a fascinating life cycle. They parasitize various arthropod species, including insects. *Cordyceps* spp. are known for their teleomorph state, where they produce fruiting bodies that emerge from the host insect's body (Gul et al. 2014).
- *Isaria* (formerly known as *Paecilomyces*): *Isaria* species are also part of the order Hypocreales. They share similar features with *Beauveria* and *Metarhizium*, making them valuable for pest management (Gul et al. 2014).

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- *Hirsutella*: *Hirsutella* fungi are entomopathogens that attack insects. They are part of the Hypocreales order and contribute to biological control strategies (Gul et al. 2014).

- *Nomuraea*: This fungus, found in the Hypocreales order, is another entomopathogen. It has been studied for its potential as a biopesticide (Gul *et al.* 2014).

- *Entomophthora, Zoophthora, Pandora,* and *Entomophaga*: These fungi belong to the order Entomophthorales (Zygomycota). They also play a role in controlling insect pests (Gul et al. 2014).

More than 750 species of fungi have been identified as entomopathogen, and many commercial mycoinsecticides are being developed. Numerous biopesticides utilizing *Metarhizium, Beauveria, Paecilomyces, Isaria*, and *Lecanicillium* species are currently being utilized globally. Given the broad spectrum of action of these fungi, they have the capacity to infect a wide variety of arthropod species (Khan et al. 2012).The most popular strain of these fungus are potential entomopathogens to wide range of insect pests from the orders Coleoptera, Lepidoptera, Orthoptera, and Diptera. EPF play a crucial role in sustainable pest management strategies. Their versatility as biological control agents allow them to target diverse hosts, making them valuable tools for integrated pest management.

In addition to managing arthropod pests, soilborne EPF also interact directly with plants and other microorganisms. These fungi colonize plant tissues and grow inside the plants as an endophyte. Endophytic EPF grow as hyphae and do not produce spores. When EPF are applied to soil, they establish a symbiotic relationship with plant roots similar to mycorrhiza. This association assists plants in coping with biotic stresses and enhances nutrient uptake

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(Dara et al. 2019).Successful endophytic colonization of plants offers numerous benefits, such as promoting plant growth, protecting against insect pests, inducing systemic resistance, antagonizing plant pathogenic fungi, bacteria, and nematodes, and mitigating the negative effects of abiotic stressors on host plants.(Vega, 2018, Khan et al., 2012; Vega et al 2009)

Entomopathogenic fungi has always been a promising and environmentally friendly alternative for managing insect pests in agriculture (Deka et al. 2021). The utilization of EPF against insect pests offers numerous advantages, including host specificity, minimal impact on non-target organisms, cost-effective mass production, high reproductive capacity, target-specific activity, short generation time, and eco-friendliness. Understanding the mechanism of action of EPF is essential for their effective use as biocontrol agents in Integrated Pest Management (IPM) strategies.

So far, no information exists on rearing of litchi insect pest on natural and artificial diet in laboratory conditions. This information is vital for studying pests' biology and bioassay of EPF against test insect.

Therefore, the present studies focus on the potential of entomopathogenic fungi as a sustainable solution for managing insect pests of litchi. The study will involve isolation, identification, and characterization of entomopathogenic fungi with a focus on their ability to combat major pests of litchi. This will involve bioassay, mass production and field application of virulent strain of EPF on major litchi pest. The outcomes of this study will lead to the development of environmental friendly alternative promoting a more sustainable approach to managing insect pests in litchi production. This will improve environmental

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health, reduce pesticides use, and conserve natural resources. For the first time, novel information on rearing insect pest of litchi in laboratory condition will be generated.

Review of Literature

Litchi (*Litchi chinensis* Sonnen) is one of the most important evergreen subtropical fruit trees belonging to the family Sapindaceae. It has high nutritive value and source of secondary metabolites, its pericarp contains good amount of phenolics which possess antioxidant anticancer activities (Lal et al. 2023). Among biotic stresses, insect pests pose significant challenges to litchi production. Approximately 42 insects and mite species has been reported to attack litchi trees and fruits at different stages of growth and fruiting. Borers, bugs, leaf rollers, leaf folder, loopers, weevils, bark-eating caterpillar (*Indarbela tetraonis* Moore) and mites are major group of pests affecting litchi production(Kumar et al. 2014, Srivastava et al. 2019; 2020). Rajeev and Vijay (2018) reported litchi mite (*Acerialitchii* Keifer), litchi fruit borer (*Conopomorpha cramerella* Snellen) and litchi leaf roller (*Dudua aprobola* Meyrick) as major pests of litchi. Lall and Mallik (1976) reported that leaf roller damage ranged from 16.70% to 71.60%, while infestation resulted in the baring of whole shoots, ranging from 12.88% to 53.54% from August to February.

Until now, growers have predominantly relied on chemical insecticides for pest control. However, the widespread use of synthetic chemicals leads to various negative consequences such as environmental contamination, development of resistance in pests, residual toxicity, pest resurgence, and adverse effects on human health and livestock. Many commercial chemical insecticides are stable, bioaccumulate easily, and exhibit toxicity, potentially persisting in the environment for longer period (Fenik et al. 2011). Chemical

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control strategies have numerous adverse health effects, including dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine issues, as reported in various studies (Nicolopoulou-Stamati et al. 2016).

Exploring alternative strategies for pest management is crucial to minimize negative impacts on the ecosystem and human health. Biological control of insect pest offers an eco-friendly and versatile alternative for pest management and enhancing plant health and productivity. Many microbial pathogens, including parasites, predators, parasitoids, pollinators, and various beneficial microorganisms, exhibit toxic properties against target organisms without harming non-target organisms. Utilizing microorganisms such as viruses, bacteria, and fungi as biocontrol agents for insect pests could offer a promising solution, potentially causing fewer harmful effects on unintended organisms(Rastegari 2020).

Apart from other microorganism, fungi have also been recognized as potential candidates and are successfully employed in pest management programs. Entomopathogenic fungi utilized as important biocontrol agents against various pests due to their broader host range, simple production, good durability and strong virulence (Khan et al. 2012). Entomopathogenic fungi are special group of soil-dwelling microorganisms that infects and kills insects through cuticle penetration. It offers economic and environmentally friendly alternative of chemical insecticides. The primary objectives of using EPF include sustaining productivity, improving environmental health, reducing pesticide usage, and conserving natural resources. Therefore, there is a need for identification, isolation, and bioformulation of EPF to overcome these issues.

Currently, entomopathogenic fungi are classified into the divisions Chytridiomycota, Ascomycota, Deuteromycota, Zygomycota, Oomycota (Purwar and Sachan 2006). However,

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More than 750 fungal species have been identified from the insect bodies, distributed among approximately 100 different genera (Sinha et al. 2016).

EPF controlling various arthropods such as ticks, insects, and mites, with two major groups Entomophthorales and Hypocreales that playing significant roles in pest suppression. Members of the Entomophthorales are naturally occurring EPF highly host-specific. Examples include *Entomophaga maimaiga* in spongy moth, *Entomophthora muscae*, and *Strongwellsea* spp. in flies, *Neozygites fresenii*, *Pandora neoaphidis* , *Entomophthora planchoniana*, and *Conidiobolus obscurus* in aphids, and *Neozygites floridana* in mites. On the other hand, members of the order Hypocreales are generalist pathogens, infecting a variety of arthropods. Examples include *Beauveria bassiana*, *Metarhizium brunneum, Hirsutella thompsonii*, and *Cordyceps fumosorosea*. These fungi can be commercially grown on artificial media, and various biopesticide formulations based on them are available globally. Both entomophthoralean and hypocrealean fungi employ identical methods of infection (Dara et al.2024).

Previous studies have shown that *Beauveria*, *Metarhizium*, *Verticillium,Hirsutella* and *Aspergillus* spp. are potential entomopathogenic fungi to control various insect pests belonging to orders Coleoptera, Lepidoptera, Orthoptera, and Diptera (Ferron 1978).

Akmal et al. (2013) reported *B. bassiana* to be highly effective against various adult aphid species including *Rhopalosiphum padi, Brevicoryne brassicae*, *Lipaphis erysimi* and *Schizaphis graminum* under laboratory conditions. Additionally, *B. bassiana* has shown efficacy against damping-off pathogens like *Rhizoctonia solani* and *Pythium myriotylum* due to its antagonistic effects associated with endophytic colonization (Ownley et al. 2008).

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The entomopathogenic fungi *Lecanicillium lecanii* is one of the most potent fungal agents against aphids. As per (Ramanujam et al. 2017) among 10 isolates of entomopathogenic fungi assessed in field trials in Karnataka, *L. lecanii* demonstrated significant effectiveness against cowpea aphids (*Aphis craccivora*), resulting in a 78% decrease at a concentration of 10^8 CFU/ml and boosting yields by 32%.

Kumar and Chowdhry (2004) reported virulence of two entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against tomato fruit borer, *Helicoverpa armigera*. Ansari et al. (2004) assessed the virulence of 34 isolates belonging to the genera *Metarhizium, Beauveria*, and *Paecilomyces* on third instar larvae of the June beetle, *Hoplia philanthus* Fiiessly, in Belgium. They observed that two isolates of *M. anisopliae* caused the highest mortality, reaching up to 90 percent after 10 weeks post-inoculation. Studies by Singh and Prakash (2012) had shown larvicidal property of *Aspergillus niger*against the mosquito vector of dengue, malaria, and filarial. Insecticidal property of *Aspergillus niger* was also reported against green bottle fly, *Chrysomyachloropyga* larvae by Essien (2005).

Increasing the resistance of entomopathogenic fungi to environmental challenges like heat, UV radiation, and dry conditions was tried. For example, Hajek et al. (2021) found that *M. anisopliae* could potentially acclimate to high temperatures through gradual exposure over multiple generations. Oliveira et al. (2018) documented that the temperature tolerance of fungus was improved through the application of emulsifiable oil-based formulations. Sahayaraj and Namachivayam (2011) noted that an oil-based formulation of B. bassiana resulted in a 72% mortality rate among *S. litura* larvae, significant increase in yield observed in Tamil Nadu.

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Recent studies have highlighted the various entomopathogenic fungal species as natural colonizers or endophytes in a wide range of economically significant crops. These include maize, coffee, potato, tomato and chickpea. Besides direct pest control, EPF as endophytes offer advantages such as suppressing pest populations, antagonizing plant pathogens and nematodes, imparting drought and salt tolerance, enhancing nutrient uptake, and promoting plant growth(Chowdhury et al. 2024; Liu et al. 2022).

EPF-based biopesticides support integrated pest management (IPM) and aid in insecticide resistance management(Ahmad et al. 2020; Liu et al. 2022). Entophytic EPF has no negative effects on the growth and development of plants rather, they are stimulative in nature (Vega 2018). Posada et al. (2007) documented the ability of *B. bassiana* to colonize coffee seedlings as an endophyte following foliar sprays, stem injections, and soil drenching with fungal spores. They further illustrated the capacity of the *Beauveria* to distribute itself within the plant, as evidenced by successful recovery from sites distant from the initial site of inoculation. Ahmad et al. (2020) reported that endophytic colonization of maize plants by *Metarhizium robertsii* promoted plant growth and altered defense gene expression in maize, and suppressed growth rate of black cutworm larvae (*Spodoptera frugiperda*). Altaf et al. (2023) reported that the endophytic colonization of B. bassiana and M. anisopliae in maize plants negatively impacted the survival, development, and reproduction of the fall armyworm (*Spodoptera frugiperda.* Sammaritano et al. (2021) investigated the potential dual effects of EPF *Metarhizium anisopliae*, which showed high effectiveness in infecting and killing various developmental stages (larvae, pupae, and adults) of the insect pest *Lobesiabotrana* under both in vitro and in vivo conditions. Additionally, these same strains of the

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entomopathogenic fungus (EPF) demonstrated the ability to inhibit the growth of the phytopathogen *Eutypella microtheca* in vitro.

Liao et al., (2017) first time reported that fungal entomopathogen *Metarhizium robertsii* produced plant growth regulator known as indole-3-acetic acid (IAA). Additionally, other strains of *Metarhizium* and *Beauveria* have been found to produce IAA, and these novel finding provide evidence that fungi have diverse ecological functions, including a significant potential to enhance plant growth and referred the term "fungal entomopathogens (Vega et al., 2009)

Apart from the benefits mentioned earlier for EPF/endophytes, fungal endophytes have gained increased attention in recent decades, along with broader biotechnological and industrial relevance, owing to their unique capacity as sources of secondary metabolites. Their ability to produce novel biochemical compounds arguably gives them an advantage over chemical pesticides. Moreover, they serve medical and pharmaceutical purposes as antimicrobial, antidiabetic, antitumor, and immunosuppressant agents(Gouda et al. 2016).

Mass production of entomopathogenic fungi is crucial for their effective utilization in pest management programs. Various strains of a fungus exhibit different growth patterns on specific media (Kleespies and Zimmermann 1992), and the choice of medium can also influence their virulence. The type of medium employed to culture the fungus often plays a significant role in determining the spore yield. Sahayaraj et al. (2008) assessed a range of agricultural products and by-products, including grains, vegetable wastes, seeds, rice husk, sawdust, as well as liquid media such as coconut water, rice wash water, and cooked rice water, for the large-scale cultivation of three entomopathogenic fungi: *B. bassiana*, *P. fumosoroseus*, and V. lecanii. They observed the highest spore production for B. bassiana on

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wheat, while sorghum resulted in the maximum spore production for *P. fumosoroseus* and *V. lecanii*. Easwaramoorthy et al. (2002) developed a method for large-scale cultivation of three entomopathogenic fungi, namely *Beauveria brongniartii*, *B. bassiana*, and *Metarhizium anisopliae*, utilizing molasses yeast media.

Rearing of litchi insect pest on natural and artificial diet in laboratory condition has not yet been tried which is vital for studying pests' biology and bioassay. This novel information needs to be generated.

Objectives

The objectives of the research studies include:

1. Collection of infected insect cadavers from litchi growing districts of Bihar and identification of entemopathogenic fungi (EPF).
2. Laboratory rearing of important litchi insect pests on synthetic and natural diets.
3. Bioassay studies of EPF against important litchi pests to select virulent isolates and Mass production for field application.
4. Biochemical change in trees and histopathology of target insects following EPF application.

Plan of work and methodology

A. Survey for infected larva collection and isolation of EPF:

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- Infected larva collection from different Litchi orchards of Muzaffarpur, Darbhanga, Samastipur and Motihari District of Bihar
- Isolation and purification to obtain pure culture of entomopathogenic fungi
- Maintenance of the pure isolates of EPFs
- Study of colony morphological and taxonomic characteristics through microscopy
- Molecular characterization of the isolated EPFs

B. Laboratory rearing of important litchi insect pests on synthetic and natural diets:

- Rearing trials on various combination of natural and artificial diets and data recording
- Rearing of *Conopomorpha sinensis* in laboratory in rearing cage (Siraj et al. 2023)

C. Bioassay studies of EPF against important litchi pests to select virulent isolates and Mass production for field application:

- Pathogenicity test proving Koch postulates for EPFs against different test insect
- Determination of lethal concentration (LC_{50} value) and lethal time (LT_{50} values)
- Microscopic studies
- Field application and recording data on efficacy in controlling various insect pests under field condition
- Mass production of entomopathogenic fungi (Kumar and Chowdhry 2004, Swapan et al. (2014)

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- Field application and recording data on efficacy in controlling various insect pests under field condition

D. Biochemical change in plants and histopathology of target insect following EPF application:

- Assay of various biochemical in leaf tissues tissue and fruits post application of EPFs
- Studying histological changes in dead insect/ larvae following EPF application
- Data analysis and communication of the result for wide distribution

Significance of the Study

Litchi is a valuable fruit crop of Bihar that faces various pest problems. The study aims to explore the potential of entomopathogenic fungi (EPF) as a bio-rational approach to manage major pests of litchi, such as the litchi fruit borer, the litchi stink bug, litchi looper and flower webber. These pests cause significant losses in yield and quality of litchi fruits, and pose a threat to the livelihoods of farmers. The study will contribute to the existing knowledge on the diversity, identification, and molecular characterization of EPF from litchi ecosystems in Bihar, India. The study will also provide new insights into the efficacy, and mass production of EPF as a biological control agent against litchi pests. The study will have practical significance for the development and implementation of sustainable and eco-friendly pest management strategies for litchi cultivation, which can reduce the reliance on chemical pesticides and enhance the environmental and human health.

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Expected Outcomes

- Identification and selection of the most virulent isolates of EPF for litchi pest management.
- Laboratory rearing of important litchi insect pests on synthetic and natural diets
- Development and testing of a cost-effective and feasible mass production technique for the selected EPF isolate.
- Field application and impact assessment of the selected EPF isolate on the litchi trees and pests.
- Biochemical and histopathological data of litchi and the target insects following EPF application.
- Insights into the potential benefits of entomopathogenic fungi on litchi yield and fruit quality.

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